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Storage stability of hydrolyzed palm kernel oil and red palm super olein blend and its soft-gel capsule

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Abstract

Hydrolyzed palm kernel oil and red palm super olein blend (HPRB) is a novel product with potential benefits for digestive health. This blend combines the antimicrobial properties of hydrolyzed palm kernel oil (PKO) with the antioxidant effects of red palm super olein (RPSO). However, the long-term stability of HPRB under different storage conditions is crucial to ensure its efficacy and safety. This study aims to evaluate the storage stability of HPRB in its oil form and as a soft-gel capsule. The research investigated the impact of various storage conditions on the phytonutrient content, fatty acid composition, and acylglycerol profile of HPRB. The results showed that cool and dark storage conditions is the best preserve for the phytonutrient content of HPRB. The fatty acid and acylglycerol compositions of HPRB remained stable regardless of the storage conditions. Accelerated stability testing of HPRB soft-gel capsules demonstrated excellent stability across various parameters, including physical characteristics, microbiological quality, and chemical stability. Shelf-life estimation indicated a relatively long shelf life for the soft-gel capsules under accelerated conditions. These results suggest that HPRB, particularly when stored appropriately or encapsulated in soft-gel form, has the potential for long-term stability and safe use.

[Keywords: Digestive health, monolaurin, phytonutrient, shelf life]

Introduction

Digestive health is a critical aspect of overall well-being, and the search for natural solutions to address digestive problems continues to gain momentum. Hydrolyzed palm kernel oil and red palm super olein blend (HPRB) is a novel product designed to address these concerns. This unique blend combines the therapeutic properties of hydrolyzed palm kernel oil (PKO) with the nutritional benefits of red palm super olein (RPSO), offering a promising approach to digestive health.

Hydrolyzed PKO is abundant in medium-chain fatty acids (MCFAs), notably lauric acid. Lauric acid is a precursor to monolaurin, a monoglyceride with potent antimicrobial properties. Monolaurin's effectiveness against various pathogens, including bacteria, viruses, and fungi, has been well-documented (Peedikayil et al., 2015; Seleem et al., 2018; Krislee et al., 2019; Barberis et al., 2021; Zhang et al., 2022). Its mechanism of action involves compromising the lipid bilayer of microbial cell membranes, resulting in cellular lysis (Ngatirah et al., 2022). This makes monolaurin a promising natural antimicrobial agent with potential applications in various fields, including food preservation and healthcare.

RPSO demonstrates a significant presence of carotenoids, especially β-carotene, and vitamin E, which act as potent antioxidants that defend cells against the damaging effects of reactive oxygen species (ROS), which cause oxidative stress (Fiedor & Burda, 2014; Zeng et al., 2020). Oxidative stress is implicated in various digestive disorders, such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) (Tian et al., 2017; Yuksel et al., 2017; Balmus et al., 2020; Chaturvedi et al., 2020). RPSO may promote a healthier digestive system by mitigating oxidative stress.

The combination of hydrolyzed PKO and RPSO in HPRB offers a synergistic approach to digestive health. The antimicrobial properties of monolaurin, coupled with antioxidant effects of carotenoids and vitamin E, may help to maintain a healthy gut microbiome and reduce inflammation. However, the long-term stability of HPRB, particularly under various storage conditions, is crucial to ensure its efficacy and safety.

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This study aims to assess the storage stability of HPRB in both its oil form and within soft-gel capsules. By investigating the effects of various storage conditions on HPRB's phytonutrient content, fatty acid composition, and acylglycerol profile, we aim to identify optimal storage conditions to preserve its quality and potency. The results of this study will offer significant information regarding the potential of HPRB as a long-term digestive health solution.

Materials & Methods

Materials

HPRB samples were provided by the Oleofood Laboratory at the Indonesian Oil Palm Research Institute (IOPRI) in Medan. HPRB was produced by blending hydrolyzed PKO and RPSO in certain ratio. Specifically, the hydrolyzed PKO was obtained via enzymatic hydrolysis utilizing Novozym 435 (Novozymes, Denmark). Two HPRB formulas were assessed in this study: HPRB-1 (60:40 ratio of hydrolyzed PKO to RPSO) and HPRB-2 (80:20 ratio of hydrolyzed PKO to RPSO). Halal certified gelatin was obtained from a local supplier in Medan and used for HPRB encapsulation. Analytical grade (≥99% purity) chemicals were purchased from Merck (Germany).

Storage conditions and sampling

HPRB was stored in non-light-proof bottles under three different conditions:

A. Dark, room temperature: Bottles kept in a dark room at 25-28 °C.
B. Dark, cool temperature: Bottles kept in a dark room at 15-17 °C.
C. Sunlight exposure: Bottles were exposed to sunlight, placed indoors near a window receiving direct sunlight during daylight hours (approximately 06:00 to 18:30), with an average temperature of 31-35 °C.

Twenty-four bottles were used for each storage condition. Samples were collected weekly for 24 weeks. At each sampling point, one bottle was randomly selected from each storage condition. The collected samples were analyzed for carotene and vitamin E content, fatty acid composition, and acylglycerol composition.

Encapsulation of HPRB

HPRB soft-gel capsules were prepared using a rotary die encapsulation process. The shell formulation consisted of gelatin (3 kg), glycerol (1 kg), and water (3 L). Potassium sorbate (1% w/w of total shell formulation) was dissolved in the water prior to its addition to the gelatin and glycerol. The ingredients were mixed at 90 °C for 1 hour. Subsequently, the temperature was reduced to 60 °C, and a vacuum was applied for 45 minutes to deaerate the gelatin mixture. The prepared shell formulation was then transferred to the encapsulation machine, and HPRB was encapsulated using a rotary die process. The resulting soft-gel capsules were subsequently dried.

Analysis of total carotene

Total carotene content in the HPRB sample was analyzed using MPOB p2.6 (2004) with UV-1700 spectrophotometer (Shimadzu, Japan).

Analysis of vitamin E and its isomers

Vitamin E and its isomers were quantified using an Acquity UPLC System (Waters, USA) in accordance with previous research (Rizki et al., 2022). The stationary phase was Inertsil ODS-3 column and the mobile phase was methanol-Lichrosolv at a flow rate of 1 $mL.min^{-1}$.

Analysis of fatty acid composition

The fatty acid composition of the samples was determined using Nexis GC-2030 (Shimadzu, Japan), following AOCS Ce 1a-13 method with minor modifications as described below. The GC system included a flame ionization detector (FID), an Agilent DB-23 column, and a Shimadzu AOC- 30i auto-injector. Nitrogen was used as the carrier gas at 1 $mL.min^{-1}$. Samples were introduced via a 1:50 split injection. The detector and injector temperatures were set at 260 °C. The temperature program began at 90 °C (hold time: 5 min), followed by a ramp of 7 $°C.min^{-1}$ to 208 °C (hold time: 5 min).

Analysis of acylglycerol composition

Acylglycerol composition was analyzed using GC-2010 (Shimadzu, Japan) with FID. A nitrogen mobile phase and Agilent DB-5HT column were used. Prior to analysis, samples were silylated with N –methyl – N – (trimethylsilyl)trifluoroacetamide (MSTFA). The injector was set at 325 °C, and oven temperature was set as follows: 100 °C (hold time: 1 min), 30 $°C.min^{-1}$ to 223 °C, 1 $°C.min^{-1}$ to 227 °C, and 5 $°C.min^{-1}$ to 360 °C (hold time: 10 min).

Accelerated stability test of HPRB soft-gel capsules

To assess the stability of HPRB-1 and HPRB-2 soft-gel capsules, an accelerated stability test was conducted. Capsules were stored in plastic bottles with plastic caps at 40±2 °C and 75±5% relative humidity for six months. This test was designed to simulate the climatic conditions of Indonesia (zone IV, hot and humid). This procedure aligned with the ASEAN Guideline on

Stability Study of Drug Product and the WHO Technical Report Series No. 863. Furthermore, the study adhered to principles outlined in the ICH Q1A (R2) Guideline on Stability Testing of New Drug Substances and Products, specifically addressing the requirements for stability data in climatic zones III and IV, as detailed in annex Q1F. Samples were collected at three time points: initial (month 0), month 3, and month 6.

Each sample underwent a series of physical, microbiological, and chemical analyses. Physical analyses included assessments of organoleptic properties (color, shape), disintegration time, and weight uniformity. Microbiological tests included total plate count, yeast and mold count, and tests for the presence of *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, and *Escherichia coli*. Chemical analyses were performed to determine moisture content and potassium sorbate content using LC-20 AD (Shimadzu, Japan).

Results and Discussion

Phytonutrient content of HPRB during storage

Carotenoids and vitamin E in HPRB are sensitive to temperature and light, making it crucial to understand their degradation patterns during storage. Figure 1 illustrates the changes in carotene concentration in HPRB under different storage conditions. As the figure shows, cool (15-17 °C) and dark storage (condition B) maintain carotene stability. In contrast, HPRB stored in a dark room at room temperature (condition A) exhibited up to a 50% reduction in carotene concentration from its initial value after 24 weeks. This degradation highlights the impact of increasing storage temperature on carotene stability. The most significant carotene degradation was observed in HPRB stored in open space exposed to sunlight (condition C). In addition to the effect of increased temperatures, UV rays from the sun contribute to carotene degradation through two possible mechanisms: (1) photochemical reactions causing isomerization and producing epoxides, apocarotenones, and apocarotenals; and (2) UV photo-oxidation facilitated by the presence of oxygen in the packaging bottle (Song et al., 2018; Atencio et al., 2022; Semitsoglou-Tsiapou et al., 2022; Šeregelj et al., 2022).

In general, the changes in vitamin E concentration during storage followed a similar trend to the changes observed in carotene (Figure 2). The rate of vitamin E degradation was slower in the dark and cool storage method (B) compared to the dark storage method at room temperature (A). However, the degradation rate of vitamin E was quite extreme in storage method C. HPRB-1 and HPRB-2 stored in open space and exposed to sunlight lost all vitamin E within a short period (17 weeks for HPRB-1 and 7 weeks for HPRB-2).

A closer look reveals that tocopherols are more susceptible to degradation than tocotrienols. Unlike tocopherols, tocotrienols have three double bonds in their isoprenoid side chain, a characteristic that contributes to their increased oxidative stability (Shahidi & De Camargo, 2016). Among the tocotrienol isomers present in HPRB, delta-tocotrienol showed the best stability during storage, while alpha-tocotrienol showed the opposite performance.

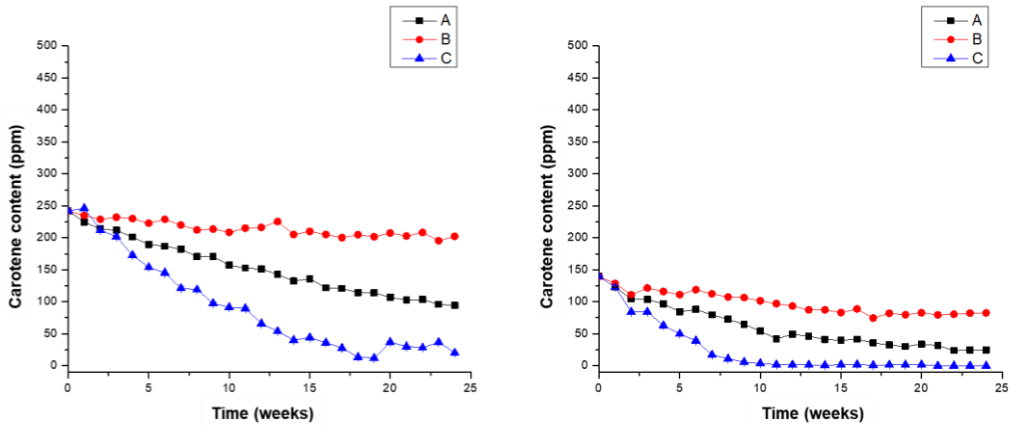

Figure 1. Changes in carotene content of HPRB-1 (left) and HPRB-2 (right) during storage under different conditions: (A) dark room at room temperature, (B) dark room at 15-17°C, and (C) open space exposed to sunlight

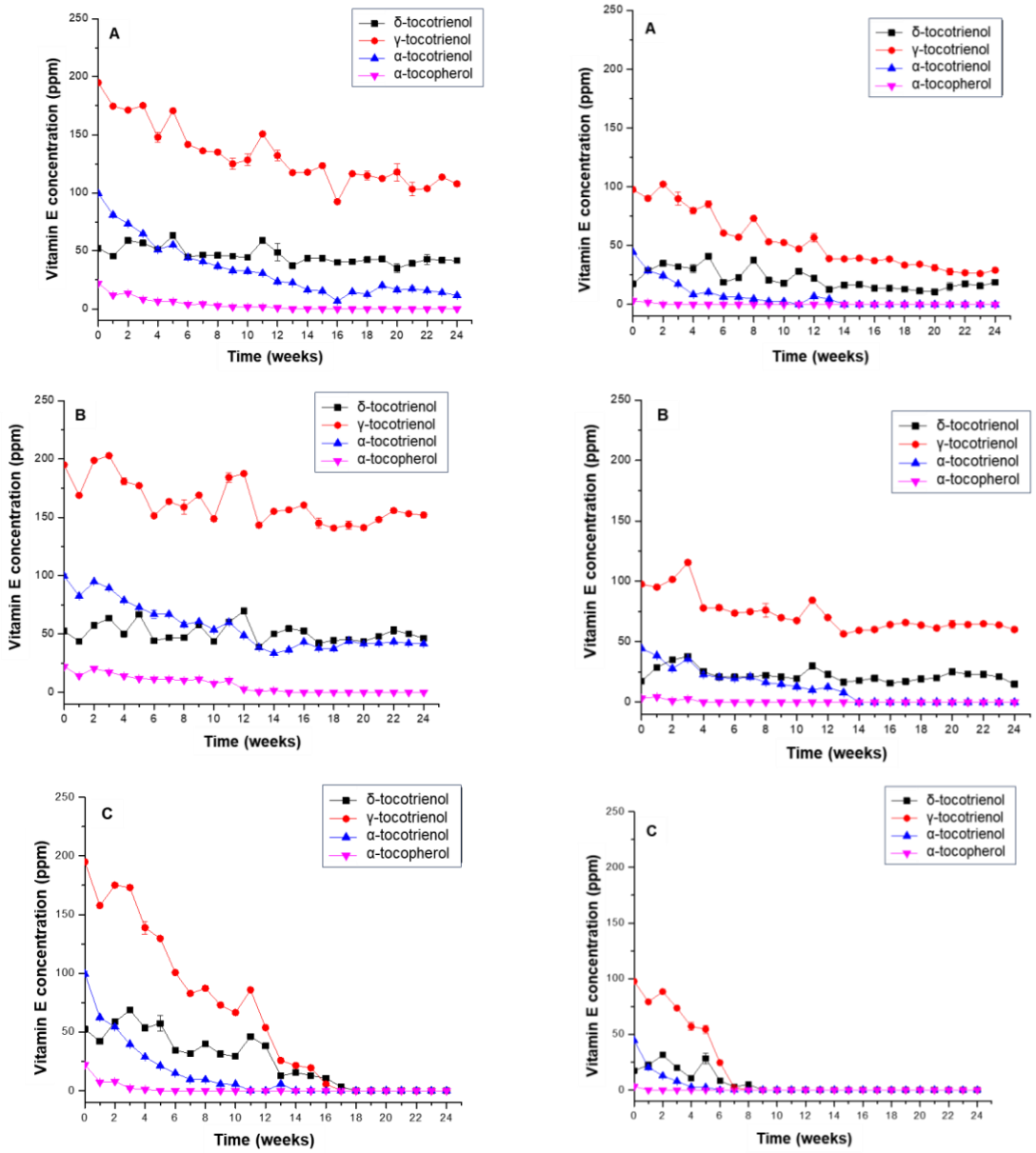

Figure 2. Changes in vitamin E isomers concentration of HPRB-1 (left) and HPRB-2 (right) during storage under different conditions: (A) dark room at room temperature, (B) dark room at 15-17°C, and (C) open space exposed to sunlight.

Changes in macro-structural components of HPRB during storage

The stability study also examined the macro-structural components of HPRB, specifically fatty acid and acylglycerol profile. Table 1 shows that HPRB's fatty acid composition includes medium-chain fatty acids (MCFA) from PKO and long-chain fatty acids (LCFA) from RPSO. The stability tests revealed no substantial alterations in the fatty acid profile for either HPRB-1 or HPRB-2 during storage (Figure 3).

Table 2 shows that the MCFA in HPRB, originating from PKO, are predominantly found as monoacylglycerols (MAG) and diacylglycerols (DAG) e.g. 1-monolaurin (La--) and 1,3-dilaurin (La-La). Conversely, the LCFA derived from RPSO are mainly present as triacylglycerol (TAG) and free fatty acids (FFA), e.g. 1,3-dipalmitoyl-2-oleoylglycerol (POP), 1,2-dioleoyl-3-palmitoylglycerol (POO), and palmitic acid. This acylglycerol composition leads to instability in TAGs and FFAs, while MAGs and DAGs demonstrate good stability during storage. This can be seen in Figure 4, FFAs and TAGs show an up-and-down trend over 24 weeks of storage, while MAGs and DAGs show better stability. This stability is influenced by the saturation and length of the fatty acid chains. Shorter, saturated chains are generally more stable than longer, unsaturated chains. Furthermore, the acylglycerol stability in HPRB was not affected by the storage condition. It can be seen in Figure 4 that graphs of storage condition A, B, and C are approximately overlapped with each other.

Table 1. Fatty acid composition of HPRB-1 and HPRB-2[a]

Fatty acid composition (%)	HPRB-1	HPRB-2
C6:0	0.13± 0.00	0.18± 0.01
C8:0	1.84±0.01	2.43±0.01
C10:0	1.75±0.00	2.39±0.01
C12:0	27.59±0.09	37.96±0.16
C14:0	9.43±0.02	12.82±0.03
C16:0	20.31±0.04	14.51±0.08
C16:1	0.07±0.00	0.04±0.00
C18:0	3.13±0.03	2.72±0.01
C18:1	27.42±0.05	22.68±0.04
C18:2	5.86±0.02	3.85±0.03
C18:3	0.12±0.00	0.06±0.00
C20:0	0.22±0.01	0.17±0.00
C20:1	0.09±0.01	0.08±0.00
ΣSFA	73.17±0.20	64.41±0.32
ΣMUFA	22.80±0.39	29.58±0.63
ΣPUFA	3.91±0.44	5.98±0.65

[a]Mean±standard deviation (n=3).
SFA=saturated fatty acid, MUFA=monounsaturated fatty acid, PUFA=polyunsaturated fatty acid

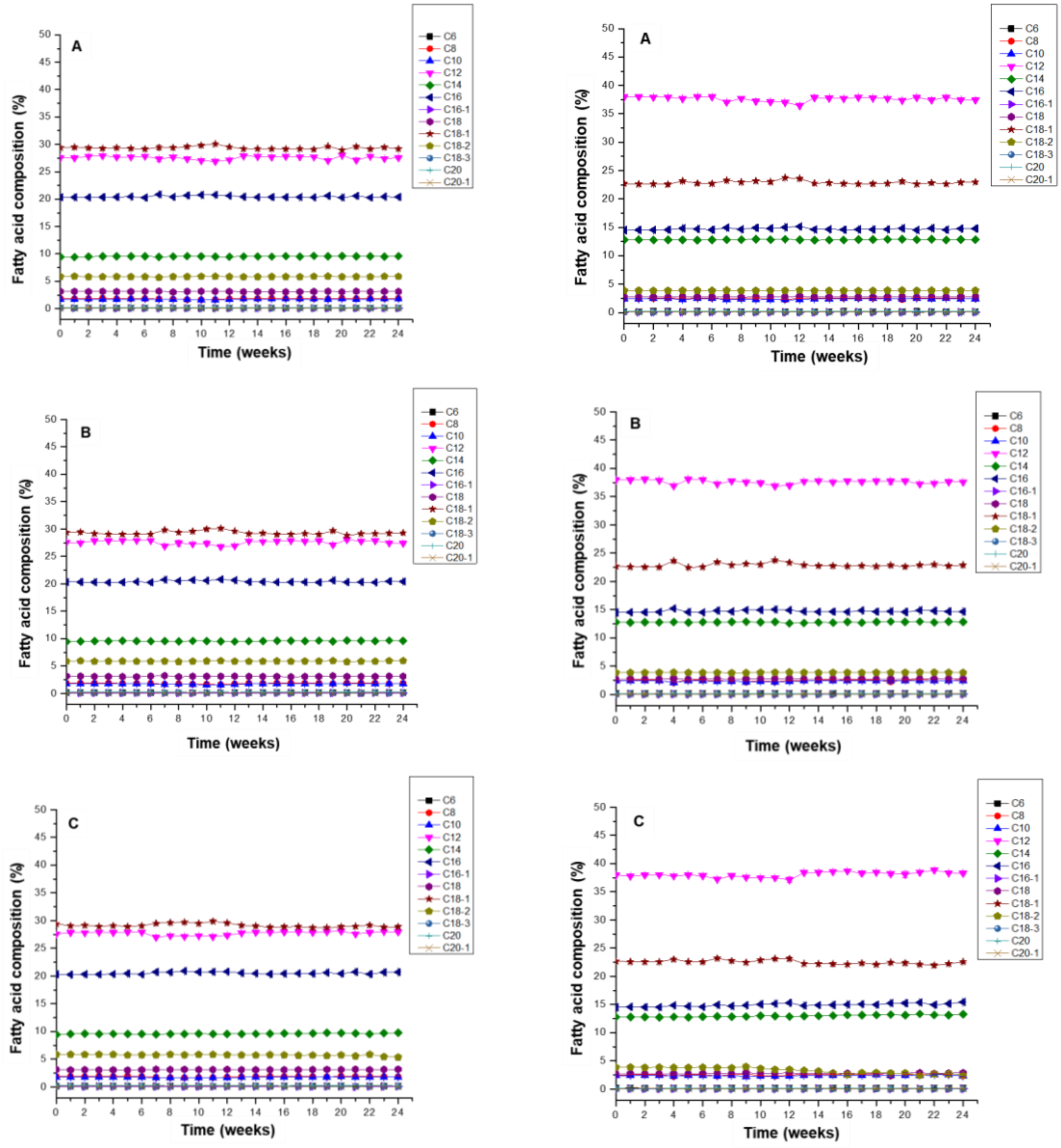

Figure 3. Changes in fatty acid composition of HPRB-1 (left) and HPRB-2 (right) during storage under different conditions: (A) dark room at room temperature, (B) dark room at 15-17°C, and (C) open space exposed to sunlight

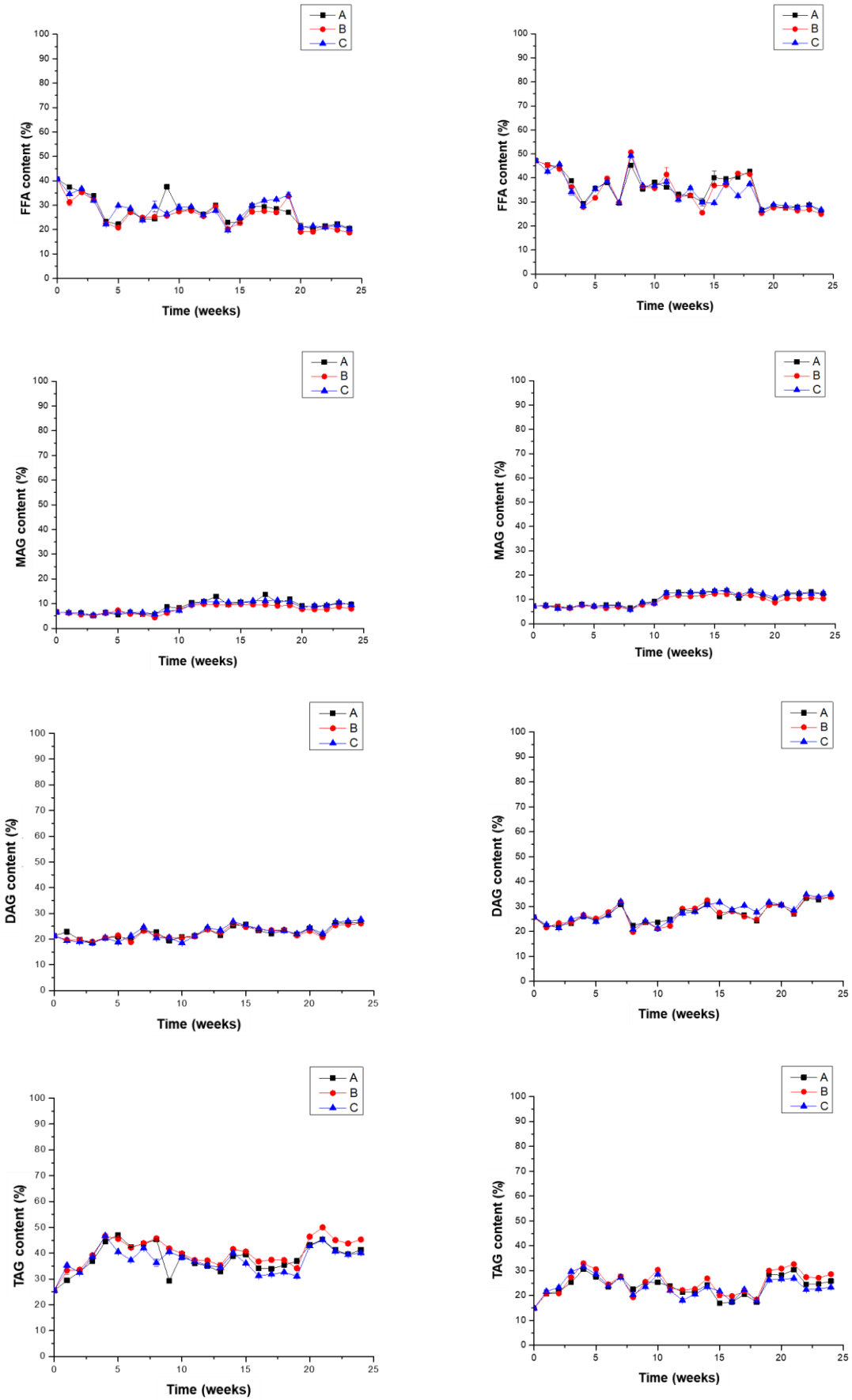

Figure 4. Changes in acylglycerol composition of HPRB-1 (left) and HPRB-2 (right) during storage under different conditions: (A) dark room at room temperature, (B) dark room at 15-17°C, and (C) open space exposed to sunlight. FFA=free fatty acid, MAG=monoacylglycerol, DAG=diacylglycerol, TAG=triacylglycerol

Table 2. Acylglycerol composition of HPRB-1 and HPRB-2[a]

Acylglycerol species[b]		HPRB-1	HPRB-2
FFA	P	6.75 ± 0.27	6.11 ± 0.30
	O	1.52 ± 0.14	3.11 ± 0.06
	S	1.36 ± 0.09	ND
	Total	**9.63 ± 0.20**	**9.21 ± 0.36**
MAG	-Ca-	ND	0.53 ± 0.06
	Ca--	ND	2.20 ± 0.59
	-La-	1.76 ± 0.39	2.30 ± 0.10
	La--	17.54 ± 0.72	24.69 ± 1.10
	-M-	ND	0.52 ± 0.03
	M--	3.61 ± 0.14	5.91 ± 0.12
	P--	1.10 ± 0.07	2.67 ± 0.24
	O--	4.70 ± 0.23	5.43 ± 1.18
	S--	0.58 ± 0.03	0.68 ± 0.16
	Total	**30.18 ± 1.57**	**44.93 ± 3.09**
DAG	La-Cp	ND	1.27 ± 1.27
	La-Ca	0.99 ± 0.05	1.75 ± 0.13
	LaLa-	2.75 ± 0.13	3.93 ± 0.01
	La-La	6.81 ± 0.01	8.90 ± 0.41
	LaM-	1.05 ± 0.01	1.78 ± 0.20
	La-M	2.33 ± 0.16	3.20 ± 0.61
	La-P	1.77 ± 0.01	2.46 ± 0.32
	LaO-	0.71 ± 0.07	1.12 ± 0.10
	Total	**16.39 ± 0.43**	**24.40 ± 1.94**
TAG	LaLaCp	2.18 ± 0.00	2.78 ± 0.02
	LaLaLa	2.10 ± 0.30	2.57 ± 0.75
	LaLaM	1.78 ± 0.35	1.83 ± 0.01
	LaLaP	0.65 ± 0.01	1.07 ± 0.18
	LaLaO	0.64 ± 0.02	0.47 ± 0.47
	LaMO	ND	0.30 ± 0.30
	PPP	0.57 ± 0.20	0.26 ± 0.2
	POP	9.53 ± 0.06	3.68 ± 0.40
	POO	20.57 ± 1.77	7.90 ± 1.02
	OOO	4.89 ± 0.09	0.59 ± 0.59
	Total	**43.80 ± 2.21**	**21.46 ± 0.78**

[a]Mean±standard deviation (n=3).
[b]Some acylglycerols might be undetected due to limit of detection.
Cp=caprylate (C8:0), Ca=caprate (C10:0), La=laurate (C12:0), M=myristate (C14:0), P=palmitate (C16:0), O=oleate (C18:1), S=stearate (C18:0), ND=not detected

Accelerated stability test of HPRB soft-gel capsules

Accelerated stability test of HPRB-1 and HPRB-2 soft-gel capsules demonstrated excellent stability across various parameters (Table 3 and Table 4). Both formulations maintained their physical characteristics throughout the six-month study period, as evidenced by the consistent shape and color of the soft-gels. While a slight increase in disintegration time was observed over time for both HPRB-1 and HPRB-2, the values remained within acceptable limits. This indicates that despite slight changes, the soft-gel shells still disintegrated efficiently enough to release the active ingredients within the expected timeframe. The weight uniformity of both HPRB-1 and HPRB-2 capsules remained consistent, ensuring accurate dosage throughout the shelf life.

The microbiological quality of both formulations remained high, with consistently zero total plate counts and yeast and mold counts. This result indicates the absence of microbial proliferation that could compromise the safety and effectiveness of the product. Remarkably, tests for specific pathogenic bacteria, including *P. aeruginosa*, *S. typhi*, *S. aureus*, and *E. coli*, were negative for both HPRB-1 and HPRB-2, confirming the absence of harmful contaminants.

These findings collectively indicate that HPRB-1 and HPRB-2 soft-gel capsules possess robust stability profiles under accelerated storage conditions. The consistent physical characteristics, along with the absence of microbial growth and pathogenic bacteria, support their potential for long-term storage and safe use.

Table 3. Accelerated stability test result of HPRB-1 soft-gel capsules

No	Parameter	Unit	Test result			Product specification
			Month 0	Month 3	Month 6	
Physical characteristics						
1	Shape	-	soft-gel capsule	soft-gel capsule	soft-gel capsule	soft-gel capsule
2	Color	-	orange	orange	orange	orange
3	Disintegration time	minutes seconds	05'54"	08'05"	09'54"	≤60'
4	Weight uniformity	g	0.4830	0.4827	0.4760	0.4347 – 0.5313
Microbiological quality						
1	Total plate count	colony.g^{-1}	0	0	0	≤10^5
2	Yeast and mold count	colony.g^{-1}	0	0	0	≤10^3
3	*Pseudomonas aeruginosa*	colony.g^{-1}	negative	negative	negative	negative
4	*Salmonella typhi*	colony.g^{-1}	negative	negative	negative	negative
5	*Staphylococcus aureus*	colony.g^{-1}	negative	negative	negative	negative
6	*Escherichia coli*	colony.g^{-1}	0	0	0	≤10
Chemical stability						
1	Moisture content	%	1.18	1.43	1.53	<10
2	Potassium sorbate content	%	0.0997	0.0992	0.0981	0.10

Table 4. Accelerated stability test result of HPRB-2 soft-gel capsules

No	Parameter	Unit	Test result			Product specification
			Month 0	Month 3	Month 6	
Physical characteristics						
1	Shape	-	soft-gel capsule	soft-gel capsule	soft-gel capsule	soft-gel capsule
2	Color	-	orange	orange	orange	orange
3	Disintegration time	minutes seconds	05'35"	06'48"	07'35"	≤60'
4	Weight uniformity	g	0.4859	0.4801	0.4803	0.4347 – 0.5313
Microbiological quality						
1	Total plate count	colony.g^{-1}	0	0	0	≤10^5
2	Yeast and mold count	colony.g^{-1}	0	0	0	≤10^3
3	*Pseudomonas aeruginosa*	colony.g^{-1}	negative	negative	negative	negative
4	*Salmonella typhi*	colony.g^{-1}	negative	negative	negative	negative
5	*Staphylococcus aureus*	colony.g^{-1}	negative	negative	negative	negative
6	*Escherichia coli*	colony.g^{-1}	0	0	0	≤10
Chemical stability						
1	Moisture content	%	1.25	1.43	1.65	<10
2	Potassium sorbate content	%	0.1007	0.0998	0.0989	0.10

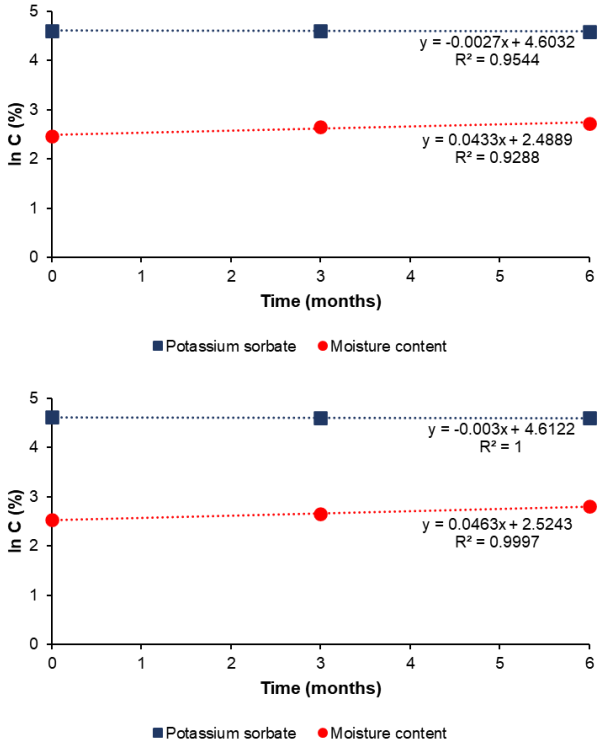

Figure 5. First-order degradation curves for potassium sorbate and moisture content changes in HPRB-1 (top) and HPRB-2 (bottom) soft-gel capsules under accelerated stability testing

Shelf life of HPRB soft-gel capsules

Shelf-life estimation of HPRB soft-gel capsules was conducted using the data of potassium sorbate degradation and changes in moisture content (Table 3 and Table 4). The data was plotted on a semi-logarithmic graph (time vs. ln C) resulting in linear equations as shown in Figure 5. These linear equations were then used to determine the rate constants (k) of a first-order kinetic model:

$$\ln C = \ln C_0 - kt$$

The k values for potassium sorbate degradation were 0.0027 and 0.0030 for HPRB-1 and HPRB-2 soft-gel capsules, respectively. Similarly, the k values for moisture content changes were 0.0433 and 0.0463 for HPRB-1 and HPRB-2, respectively. Shelf life was subsequently calculated using these k values and a 90% expiration limit (i.e., allowing for 10% degradation of potassium sorbate or a 10% change in moisture content) (Park et al., 2018; Maia et al., 2021; González-González et al., 2023).

Analysis revealed an estimated shelf life of 38 months based on potassium sorbate degradation and 46 months based on moisture content changes for HPRB-1. For HPRB-2, the estimated shelf life was 37 months and 43 months, respectively. These findings suggest a relatively long shelf life for HPRB soft-gel capsules under accelerated conditions, indicating potential stability for long-term storage under normal conditions.

Conclusion

Hydrolyzed palm kernel oil and red palm super olein blend (HPRB) stored under cool and dark conditions maintained good stability of phytonutrients, fatty acids, and acylglycerols. Accelerated stability test of HPRB soft-gel capsules demonstrated excellent stability across various parameters, including physical characteristics, microbiological quality, and chemical stability. HPRB-1 soft-gel capsules could remain stable for approximately 38 to 46 months, while HPRB-2 capsules were projected to maintain their quality for roughly 37 to 43 months, based on potassium sorbate degradation or moisture content as the determining factor. The results suggest that HPRB, particularly when stored appropriately or encapsulated in soft-gel form, has the potential for long-term stability and safe use.

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